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Antifungal treatment in sarcoidosis—A pilot intervention trial

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Summary
Background: Sarcoidosis is generally treated with corticosteroids that are not always an effective therapy.
Objectives: To assess if treatment with antifungal drugs would improve the clinical status of patients with sarcoidosis.
Methods: Patients ($n = 18$) with sarcoidosis grades II and III according to established criteria and without clinical and immunological signs of fungal infection, were treated with antifungal medication together with corticosteroids for 3–6 months. Pulmonary X-ray infiltration, lung function, and severity of symptoms were registered before and after the treatment and at follow up 9–58 months later.
Results: The treatment resulted in statistically significant decreases in the degree of pulmonary infiltration with an average decrease in the group from 2.0 to 1.0. There were also significant increases in diffusion capacity and decreases in the severity of symptoms.
Conclusion: It is suggested that treatment with antifungal drugs may be useful, at least in certain cases of sarcoidosis.

Introduction

In spite of extensive research efforts over many years, the aetiology of sarcoidosis remains an enigma.[1] Recent reviews have demonstrated that mycobacteria as well as other micro-organisms remain primary suspects although no convincing evidence is available.[2,3] A few clinical and epidemiological studies support a role for mould exposure. In a case control study using questionnaires, cases of sarcoidosis were found to have a more frequent exposure to fungi at the workplace and to moisture problems at home than controls, but the response rate was very low both among cases and controls.[4] Occupational risk factors were studied among 273 cases of sarcoidosis and 618 siblings without the disease.[5] Specific exposures that were associated with the disease and imply possible fungal exposures were vegetable dusts, and indoor exposure to high humidity,

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water damage or musty odours. In a case-control study on 706 newly recruited cases of sarcoidosis and equal numbers of age-, race-, and gender-matched controls, there were positive associations between sarcoidosis and agricultural employment, and work environments with mould/mildew exposure.[6]

One of the authors (MT) made a clinical observation that the treatment of fungal foci (candida vaginitis and sinusitis) present in three patients with sarcoidosis brought about an unexpected improvement in lung X-ray findings. To further assess this finding, a pilot intervention trial was undertaken with the hypothesis that treatment of cases with sarcoidosis with antifungal medication would improve their clinical condition.

Material and methods

Patients

The clinical material comprised patients with sarcoidosis diagnosed at the University hospital in Ljubljana. The patients for the study ($n = 18$) were selected from those with a stage II or III disease with poor or no clinical regression after at least 6 months of treatment with corticosteroids (prednisolone 12 or 16 mg every second day) or patients with a relapse after discontinuation of treatment. Inclusion criteria were X-ray-diagnosed bilateral parenchymal infiltrates corresponding to stages II and III according to the ATS classification.[7] Lung biopsies were taken from all patients. The presence of non-caseating granulomas was verified histologically and all biopsies were stained and about half of them were cultured to exclude the presence of fungal infection. No biopsies were taken after the corticosteroid treatment prior to antifungal treatment.

Examinations

The X-rays comprised standard front views of the chest in erect position, at 150 kv and 200 cm focus–film distance, automatic exposure in deep inspiration (Ferrania Chest GbB 7700 501 films with standard automatic developing). There was no evidence of aspergillomas on X-ray or CT scanning before antifungal treatment (cavities, pleural thickening or other findings indicative of aspergillosis).

The X-rays were scored for extent of infiltration (0 = normal, 1 = ca 25% of lung field involved, 2 = up to 50%, 3 = up to 75%, and 4 = virtually the whole lung field involved) by two experienced radiologist and four residents not knowing the patient's identity, diagnosis or the date of X-ray examination. There was a disagreement between the groups for two X-rays and these were assigned according to the less favourable score.

Measurements were made of forced expiratory volume in 1 s (FEV_1), vital capacity (VC) and lung diffusion (DLCO, $n = 17$) according to the ATS criteria and expressed as % of expected. Serum angiotensin converting enzyme (SACE) was measured in serum using standardised methods. Immunological examinations were made before and after antifungal treatment and comprised fungal IgG, IgM, and IgA antibodies in serum but antibody titre was not used to judge the efficiency of the treatment.

Information on the presence of symptoms was obtained in structured interviews before and after the treatment, using a numerical scale for severity of cough (1 = mild, 2 = moderate, 3 = severe, every day, 4 = severe, day and night), dyspnoea (1 = on heavy exercise, 2 = on moderate exercise, 3 = on minimal exercise, 4 = at rest), chest pains (1 = little pain, 2 = moderate pain, 3 = severe pain, 4 = very severe pain), malaise (yes/no), and increase in body temperature ($>37\,^{\circ}C$).

Treatments

The patients were treated with antifungal drugs—Itrakonasol, Fluconasol, and Ketonazol (all at 200 mg/day) together with corticosteroids in the usual dose. At 3–6 months, after initiation of antifungal treatment, the dose was decreased to 4–6 mg. The choice between the drugs was made based on the presence of antibodies to *Aspergillus* or *Candida*. If no clear differences in titre were present, Flukonasol was chosen. Liver function was tested before and every second week throughout fungal treatment. No indications for discontinuation of treatment were found. One patient discontinued antifungal treatment after 2 months by her own and was excluded from the material. A summary of the patient characteristics is presented in Table 1.

The duration of corticosteroid treatment before the intervention ($n = 11$) was 26 months (range 14–48 months). Seven of the patients had been without corticosteroid treatment for 2–36 months when they had a relapse in their disease. The antibody levels were below those generally considered to reflect an ongoing fungal infection.

Treatment of data

For each individual, the difference before and after treatment was calculated for X-ray readings, lung function and severity of symptoms. The presence of positive values (deterioration) or negative values (improvement) before and after treatment were tested for significance using Wilcoxon's signed rank test, with $P < 0.05$ as the critical value for statistical significance.

Follow-up

A follow-up comprising X-ray was made 9–58 months after cessation of antifungal treatment.

Results

The results from the X-ray scores at the time of diagnosis of sarcoidosis, after corticosteroid treatment and after antifungal+corticosteroid treatment are seen in Table 2.

There were no significant differences in scores between the time at diagnosis and after corticosteroid treatment. The difference before and after antifungal+corticosteroid treatment was significant ($P < 0.001$). One patient improved 2.5 points, two 2 points, three 1.5 points, three 1 point, seven 0.5 point and two were unchanged. Sixteen persons had scores in excess of 1 before treatment as compared to five after treatment.

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Table 1 Background characteristics of patients.

Age	Gender	Disease stage	Corticosteroid treatment		G titres units/ml		Drug
			A	B	*Aspergillus*	*Candida*	
42	F	2	32	—	0	< 30	K
34	F	2	27	—	70	ND	I
37	F	2	14	—	<30	<30	I
43	M	2–3	17	—	<30	<30	F
50	M	2	—	10	<30	0	I
41	M	2–3	36	—	18?	61	I
44	F	2	18	—	39	42	F
48	F	2–3	36	—	74	<30	F
41	M	2	48	—	175	9	I
42	F	2–3	—	5	25	<30	I
44	M	2	—	9	<30	<30	F
57	M	2	24	—	77	172	I
34	M	2–3	—	2	<30	<30	F
40	F	2	—	17	90	133	F
51	F	2	19	—	49	60	F
59	F	2	—	36	<30	<30	I
67	F	2–3	—	10	35	40	F
31	M	2–3	17	—	<30	<30	I

Corticosteroid treatment: A = months treatment before antifungal treatment was initiated, B = months without treatment before relapse of disease and start of antifungal treatment. Treatment with Flukonasol (F), Itrakonasol (I) or ketonazol (K).

Table 2 X-ray scores of pulmonary infiltration among patients at diagnosis of sarcoidosis (A), after corticosteroid treatment (B), after antifungal +corticosteroid treatment (C) and at follow-up (D).

Subject	A	B	C	D
1	2.5	2.5	1.0	0
2	2.0	3.5	1.0	0
3	2.5	2.5	1.0	0
4	3.0	2.0	1.0	1
5	1.0	0.5	0.5	0
6	2.0	3.0	1.0	1.5
7	2.0	1.5	1.0	1
8	2.0	1.5	1.0	1
9	3.0	2.0	1.5	0
10	1.5	1.0	0.5	0.5
11	2.5	2.0	1.5	0
12	1.0	2.0	2.0	1
13	1.0	1.5	0.0	0
14	2.0	2.0	1.5	0
15	3.0	2.0	1.0	2
16	3.0	2.0	0.0	1
17	1.5	2.0	1.0	2
18	3.0	2.5	2.0	0.5
Mean	2.1	2.0	1.0	0.6

For DLCO, 15 out of 17 subjects improved. The mean value in the group before treatment was 86.1, SD 9.5% as compared to 92.1, 7.3% after treatment ($P = 0.022$). There were no significant differences in FEV_1 or VC before and after the treatment (data not shown). There were no significant differences in SACE values before and after treatment.

Regarding symptoms, the severity of cough was lower after antifungal treatment as compared to before among 12 persons ($P = 0.003$). Dyspnoea was lower among 14 ($P = 0.001$) and chest pains were lower among 13 ($P = 0.001$). Seven persons had persistent fever (>37°) before the treatment compared to none afterwards.

At the follow-up, 15 patients had been without corticosteroid treatment for 6–55 (average 16) months. Nine patients had a lower score than at cessation of antifungal treatment (Table 2), four were unchanged and four had increased scores. Figure 1 presents the average X-ray score at different times during the investigation.

Discussion

The results from the study support the initial observation that treatment with antifungal medication improved the clinical and subjective signs of sarcoidosis among the majority of patients studied.

There are, however, some methodological aspects that need to be considered.

The X-ray evaluation involved a subjective scoring of the extent of infiltration in a double-blind manner. There are other alternatives for grading but this was considered a robust measure and, as the results are expressed as a comparison between two readings for each patient, the result should be reliable.

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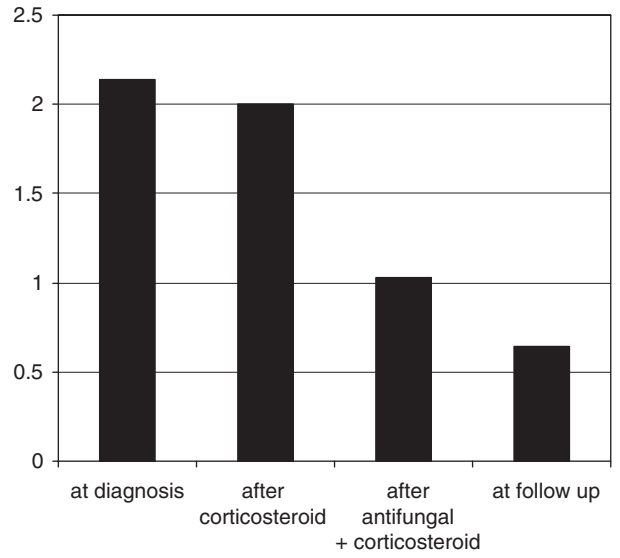

Figure 1 Average X-ray scores in relation to treatments.

The patients were by purpose selected among those who had a long-term history of the disease or who relapsed after discontinuation with the traditional corticosteroid treatment. It cannot be excluded, however, that some patients could have hade a spontaneous recovery which is a well-known phenomenon in sarcoidosis. On the other hand, experience demonstrates that spontaneous recovery is rare for patients with chronic disease of long duration. It could have occurred in some of the patients but cannot account for the overall improvement that was found after the antifungal treatment.

The antifungal medication was not administered in a double-blind design. This will be required in further studies to verify the findings from this preliminary study. It is unlikely, however, that the general improvements in lung X-ray scores were a placebo effect.

There are several possible explanations for the improvements described. It is possible that the antifungal drugs as such had some unknown effect on the immune system, causing a regression of pulmonary infiltrates and subjective symptoms. No such effects have, however, been described for the antifungal agents used. It is also possible that the antifungal drugs could have improved the efficiency of the combined treatment with corticosteroids. On the other hand, the treatment with corticosteroids before the antifungal treatment comprised treatment with optimal doses without clinically significant results in 11 of the patients in the study.

A challenging possibility is that fungi present in the patients could have been of importance. Support for the hypothesis that fungi may be a risk factor in sarcoidosis is found in two recent case-control studies cited earlier.[5,6] Evidence that fungi may cause inflammation in the lungs is found in a study on 11 patients with late-onset asthma and a positive skin prick test to species of Trichophyton.[8] They were given Fluconazole for 5 months and there was a significant decrease in airway reactivity to the fungal antigen and an improvement of the symptoms of asthma.

The low antibody titre in serum and the absence of fungi in the biopsies suggest that there was no fungal infection with active growth in the patients. If the causative agent in the sarcoidosis patients that improved after antifungal treatment were the presence of fungi in the lung or other organs, the disease mechanism could be a late hypersensitivity reaction, mediated by denditric cells and T-cell lymphocytes.[9,10] The fungal cell wall component $(1\rightarrow3)$-β-D-glucan could be important for such reactions.[11,12] This agent affects lymphocytes and macrophage function and causes granulomas in the lungs.[13] Some data suggest that it induces a Th2-like response[14,15] which could explain why antibodies to a variety of different micro-organisms are increased in patients with sarcoidosis.[3]

Molds and $(1\rightarrow3)$-β-D-glucan have also been implied in the pathogenesis of another granulomatous lung disease—hypersensitivity pneumonitis (HP). Sarcoidosis and HP share some pathological characteristics and both diseases are less common among smokers.[16] In this material, none of the cases were smokers as compared to a 35% prevalence of smoking in the Slovenian population. HP is also accompanied by general symptoms such as fatigue and weight loss, similar to the condition often present among patients with sarcoidosis.[17]

In conclusion, the results from this pilot, clinical intervention study suggest that treatment with antifungal drugs could comprise a useful addition to the conventional treatment with corticosteroids in certain cases of sarcoidosis. Further intervention studies are required to assess the general validity of this conclusion.

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Emina Hadzibajramovic performed the statistical analysis.

Ethical approval: The Ethical committee for Medicine, Ministry of Health, Ljubljana, Slovenia approved the study with the usual regulation concerning patient's approval of participation.

Competing interests: None of the authors have any financial relations with or received funding from pharmaceutical firms manufacturing antifungal drugs.

References

1. Martin II WJ, Iannuzzi MC, Gail DB, et al. Future directions in sarcoidosis research. Summary of an NHLBI working group. *Am J Resp Crit Care Med* 2004;**170**:567–71.
2. Newman LS, Maier LA, Rose CS. Sarcoidosis. *N Engl J Med* 1997;**336**:1224–34.
3. duBois RM, Goh N, McGrath D, et al. Is there a role for microorganisms in the pathogenesis of sarcoidosis? *J Int Med* 2003;**253**:4–17.
4. Ortiz C, Hodgson M, McNally D, et al. Sarcoidosis and exposure to occupational and environmental agents. In: Johanning E, editor. *Bioaerosols, fungi and mycotoxins; health effects, assessment, prevention and control*. Albany, New York, USA: Eastern New York Occupational and Environmental Health Centre; 1999. p. 476–81.
5. Kucera GP, Rybicki BA, Kirkey KL, et al. Occupational risk factors for sarcoidosis in African–American siblings. *Chest* 2003;**123**:1527–35.
6. Newman LS, Rose CS, Bresnitz EA, et al. A case-control etiological study of sarcoidosis–environmental and occupational risk factors. *Am J Respir Crit Care Med* 2004;**170**:1324–30.

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7. Statement on sarcoidosis. *Am J Respir Crit Care Med* 1999; **160**:736–55.
8. Ward GW, Woodfolk JA, Hayden ML, et al. Treatment of late-onset asthma with fluconazole. *J Allergy Clin Immunol* 1999;**104**:146–51.
9. Ho L-P, Urban BC, Thickett DR, et al. Deficiency of a subset of T-cells with immunoregulatory properties in sarcoidosis. *The Lancet* 2005;**365**:1062–72.
10. Ota M, Amakawa R, Uehira K, et al. Involvement of dendritic cells in sarcoidosis. *Thorax* 2004;**59**:408–13.
11. Rylander R. Role of endotoxin and glucan for the development of granulomatous disease in the lung. *Sarcoidosis* 1989;**6**:26–7.
12. Rylander R. (1→3)-β-D-glucan in the environment: a risk assessment. In: Young S-H, Castranova V, editors. *Toxicology of (1→3)-β-glucans: glucans as a marker for fungal exposure*. Boca Raton, FA: CRC Press; 1995. p. 53–64.
13. Johnson KJ, Glovsky M, Schrier D. Pulmonary granulomatous vasculitis induced in rats by treatment with glucan. *Am J Pathol* 1984;**114**:515–6.
14. Wan G-H, Li C-S, Guo S-P, et al. An airborne mold-derived product, (1→3)-β-D-glucan, potentiates airway allergic responses. *Eur J Immunol* 1999;**9**:2491–7.
15. Ormstad H, Groeng EC, Lovik M, et al. The fungal wall component beta.1, 3-glucan has an adjuvant effect on the allergic response to ovalbumin in mice. *J Toxicol Environ Health* 2000;**61**:55–67.
16. American Thoracic Society, European Respiratory Society, World Association of Sarcoidosis and other granulomatous disease. Statement on sarcoidosis. *Am J Resp Crit Care Med* 1990;**160**:736–55.
17. Curtis JR, Borson S. Examining the link between sarcoidosis and depression. *Am J Resp Crit Care Med* 2001;**163**:306–8.

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